THE APPRESSORIA OF THE ANTHRACNOSES.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXXIV.

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(WITH SEVEN FIGURES)

IN describing a number of new plant diseases in 1883 FRANK[1] gave an account of peculiar spore-like organs produced by the germ tubes of spores of the bean anthracnose. He showed further that these organs acted as holdfasts, by means of which the fungus was firmly attached to its host during the early phase of development. In the same paper he described analogous organs of *Fusicladium Tremulae* and *Polystigma rubrum.* Almost simultaneously FISCH[2] described the holdfasts of Polystigma, but he did not at all recognize their true significance. He regarded them as "secondary spores" which served in the distribution of the fungus, since the ascospores are embedded in slime when ejected, and are therefore not suited for dissemination by the wind. FRANK first recognized the true nature of these bodies, and gave to all organs of this class the name *appressoria* or adhesion organs. Later MEYER[3] again described and figured the adhesion organs of Polystigma, but added no new observations. In 1886 DE BARY[4] first showed that the complex adhesion organs of Sclerotinia were produced as the result of a mechanical stimulus, but BÜSGEN[5] made the most complete study from a physiological standpoint. He showed that the germ tubes of many parasitic fungi produce adhesion organs of

[1] FRANK, B., Ueber einige neue und weniger bekannte Pflanzenkrankheiten. Ber. Deutsch, Bot. Gesells. 1:29–34, 58–63. 1883; Landw. Jahrb. 12:511–539. *pls. 3.* 1883.

[2] FISCH, C., Beitrage zur Entwickelungsgeschichte einiger Ascomyceten. Bot. Zeit. 40:851–870. *pls. 2.* 1882.

[3] MEYER, B., Untersuchungen über die Entwickelung einiger parasitischer Pilze bei saprophytischer Ernährung. Landw. Jarhb. 17:915–945. *pls. 4.* 1888.

[4] DE BARY, A., Ueber einige Sclerotinien und Sclerotinien-krankheiten. Bot. Zeit. 44:377 et seq. 1886.

[5] BÜSGEN, M., Ueber einige Eigenschaften der Keimlinge parasitischer Pilze. Bot. Zeit. 51:53. 1893.

various forms, and that their formation is due to a mechanical stimulus resulting from contact of the germ tube with some solid body.

These accounts seem to have escaped entirely the notice of American writers on the bitter rot, as is indicated by the many speculations and by the curious interpretations of the characteristic adhesion organs of the bitter-rot fungus and of other anthracnoses. The first economic account of the bitter rot appears in the *Report* of the chief of the Section of Vegetable Pathology for 1887.[6] Here the formation and germination of the appressoria are described. They are regarded as secondary spores, but no particular function is attributed to them. Excellent figures are also given on *plate 3* of the *Report* of 1890[7]. In 1891 Miss E. A. SOUTHWORTH[8] published the most complete account of the fungus up to that time. Regarding the "secondary spores" Miss SOUTHWORTH says: "What the conditions were that decided their appearance could not be determined. They were produced both in nutritive media and water, but seemed to be especially numerous where the ends of the hyphae came in contact with some hard substance like the cover-glass, and in two cases the addition of an extra drop of nutritive medium had the effect of stopping their formation." As to their function nothing is said, except that they are regarded as resting spores. (See *note*, p. 142.) In 1892 HALSTED published a short account of the secondary spores of anthracnoses.[9] He extends the list of anthracnoses which produce these organs to twenty-five species, including members of both Gloeosporium and Colletotrichum. ALWOOD[10] describes the production of "resting spores" by the bitter-rot fungus, but from his figures and description it is impossible to determine whether he had before him the bodies in question. Other writers have followed these investigators in their interpretation of the peculiar adhesion

[6] SCRIBNER, F. LAMSON, Bitter rot of apples. Rep. Sect. Vegt. Path. U. S. Dept. Agr. **1887**: 348–350.

GALLOWAY, B. T., Ripe rot of grapes and apples. *Idem.* **1890**: 408

[8] SOUTHWORTH, E. A., Ripe rot of grapes and apples. Journ. Myc. **6**: 164–173. *pl. 1.* 1891.

[9] HALSTED, B. D., The secondary spores in anthracnoses. N. J. Agr. Exp. Sta. Rep. **1892**: 303.

[10] ALWOOD, W. B., Ripe or bitter rot of apples. Agr. Exp. Sta. Va. Bull. 40. 1894.

organs of Gloeosporium. CLINTON[11] regards them as chlamydospores. They are also briefly described by VON SCHRENK and SPAULDING[12] who add *Gloeosporium cactorum* to the list of anthracnoses producing them. In order to clear up the uncertainty expressed in the literature regarding these organs, the following experiments and observations on the appressoria of *Gloeosporium fructigenum* are here recorded.

FORMATION OF APPRESSORIA.

As has been said, DE BARY and BÜSGEN have shown that the stimulus of mechanical contact is the cause of the formation of adhesion organs. Regarding the adhesion organs of Gloeosporium Miss SOUTHWORTH mentions the fact that they are especially numerous where a hypha comes into contact with some hard object like the cover glass. HALSTED finds that a rich nutrient medium produces only a meager supply of "special cells," while pure water increases their production. In neither case were these suggestions further investigated. Other writers had suggested in a general way that "unfavorable conditions" and starvation of mycelium cause the formation of the special cells.

Spores were sown in convex drops of water on slides kept in a moist chamber. Under these conditions the spores germinate rapidly, but their behavior varies according to their position in the drops. Those which sink to the bottom of the drop form a short germ tube, which enlarges into a round or pear-shaped disc when it comes into contact with the glass. In 12 to 18 hours this disc has developed into a complete adhesion organ (*fig. 1*). It is a brown spore-like body, having a thick wall which is perforated on its lower appressed surface with a very distinct germ pore. The adhesion

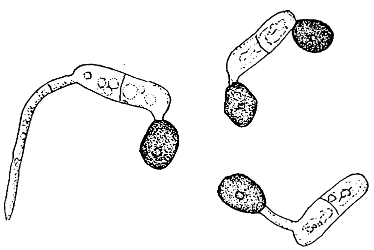

FIG. 1.—Appressoria formed by spores germinating in water on glass slides.

[11] CLINTON, G. P., Apple rots in Illinois. Univ. of Ill. Agr. Exp. Sta. Bull. 69. *pls. 10.* 1902.

[12] VON SCHRENK and SPAULDING, The bitter rot of apples. U. S. Dept. Agr. Bur. Plant Industry Bull. 44. *pls. 9.* 1903.

organs are so firmly fixed to the slide that they are not easily washed off by a jet of water. Other spores remain floating in the drops of water, being held by the surface film. These also germinate readily, but they never form adhesion discs while the germ tubes remain free in the water. Other spores were sown in drops of water placed on the surface of apples. These behaved in the same way as those on slides. Spores in hanging drops produced mostly mycelia, since very few germ tubes came into contact with the glass. The experiment was then varied by substituting beet infusion for the drops of water. The result was striking. The germ tubes produced no appressoria, but grew out into long hyphae, regardless of the fact that they were often in contact with the surface of the glass or with the cuticle of the apple. When sown in nutrient media of any kind, solid or liquid, the spores of Gloeosporium germinate and form mycelia directly.

These experiments show that the formation of appressoria is induced by a contact stimulus, but in the presence of abundant nutrient material the germ tube loses its power to react to contact stimuli, and the formation of appressoria is inhibited. If this were not the case, the mycelium would react to the contact of every obstacle, such as cell walls or starch grains, which it met in its course through the tissues, and growth would thus be made practically impossible. This is illustrated by the behavior of spores in weak beet infusion. Here the germ tube shows a tendency to form an appressorium, but before this is well formed it grows out again into a mycelial hypha, which immediately repeats the process. In old agar cultures which have been exhausted, the hyphae form a series of thick-walled cells of the nature of appressoria. These do not have the normal shape, but assume fantastically lobed forms, so closely crowded that they resemble sclerotia-like masses. The exhaustion of the nutrient material in the agar and the contact with the glass or other solid particles no doubt leads to the formation of these masses.

GERMINATION OF THE APPRESSORIA.

The appressoria germinate readily on a slide when covered with nutrient solution. The germ tube always emerges from the pore

on the surface appressed to the glass. By its vigorous growth it tilts the body to one side (*fig. 2*).

The process of penetration was observed by sowing spores on berries of *Berberis Thunbergii*, which are readily infected by the fungus, although some other species seem to be immune. From the pore on the lower flattened side of the adhesion disc, a slender tube protrudes and dissolves an arrow channel in the wax covering the cuticle. Although at first very slender, the hypha soon becomes larger and dissolves large cavities in the wax (*figs. 3, 4*). The fact that these cavities are more extensive than is necessary for the accommodation of the germ tube would seem to indicate that a solvent is secreted in sufficient quantities to accumulate on the outside of the infecting hypha. Finally the cell wall is perforated and the mycelium branches freely within the cells, at the same time sending hyphae into the neighboring cells. The penetration of the germ tube through the cuticle of the apple has frequently been observed, although it has not been possible to follow the mycelium farther, probably on account of the early collapse of the cells and the consequent

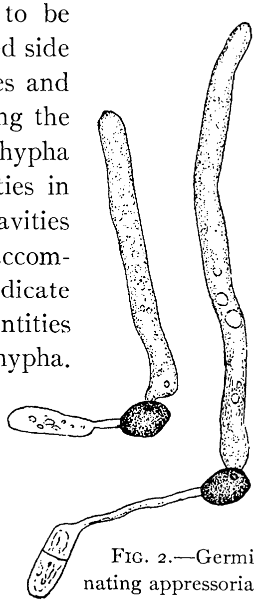

FIG. 2.—Germinating appressoria.

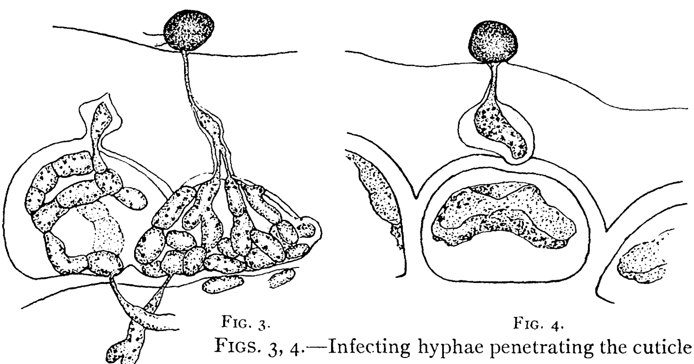

FIG. 3. FIG. 4.

FIGS. 3, 4.—Infecting hyphae penetrating the cuticle of berries of *Berberis Thunbergii*.

accumulation of débris. The channel in this case is very narrow but well defined. Contrary to former supposition no previous injury

or puncture of the fruit is necessary. This is further demonstrated by the number of infections occurring in apples. In some cases 100 to 200 infections were found on single apples, and recently SCOTT[13] reports the enormous number of 1,000 to 1,200 infections on single fruits. It is not likely that these represent previous mechanical injury to the fruits.

APPRESSORIA IN RELATION TO DISSEMINATION.

The behavior of the appressoria of the bitter-rot fungus under natural conditions is of interest from a biological standpoint. The spores of this fungus are imbedded in a gelatinous substance, which causes them to stick together in waxy masses when dry. By reason of this condition the spores cannot be distributed by wind. So far as known they are entirely dependent for their dissemination upon rain, although it is probable that insects take an active part in carrying the spores from tree to tree. Each season the first general infection of apples by the bitter rot is due to rain washing the spores from the limb cankers, in which the fungus hibernates, to the apples below. This is plainly shown by the observation that on a tree the infected apples are distributed within an area that can be circumscribed by a cone having its apex at the canker, the source of infection. Furthermore, drops of rainwater, collected from badly infected trees, usually contain numerous spores of the bitter-rot fungus.

Since the rain, at least in many cases, is the chief factor in distributing the bitter-rot spores, it is of interest to determine the effect of wetting and drying on the spores, and also the relative vitality of the spores and the appressoria. It should be stated, that while the spores are imbedded in their mucilaginous covering, they retain their vitality for a long time, but not during the entire winter, as has often been reported. In the latitude of Southern Illinois, spores remaining on apples under the trees either germinate[14] or perish long before spring. Spores taken from time to time from a diseased apple, which was kept dry in the laboratory from August until January, showed a large percentage of germination as late as Nov. 29, but later rapidly lost their vitality.

13 SCOTT, W. M., The control of apple bitter rot. U. S. Dept. Agr. Bur. Pl. Industry Bull. 93. *pls. 8.* 1906.

14 See also CLINTON, *l. c.*

To test the resistance of spores to drying after being freed from the surrounding mucilage by washing, a quantity of spores was shaken up with water and then spread out on glass slides which were allowed to dry. After remaining dry 14 hours, few spores germinated when again placed in water; after 24 hours, none germinated. At different times during the summer spores were shaken up in water and sprayed on filter paper, apples, and glass slides, but it was impossible to cause them to germinate after having been dried 24–30 hours.

That the appressoria are more resistant is shown by the following experiment. Appressoria were produced by sowing spores in drops of water on slides which were kept in a moist chamber until the following day. The slides were then allowed to dry, all the submerged spores having produced appressoria. The germination of the appressoria was tested from time to time by covering a slide with sugar-beet infusion. The appressoria germinated, though irregularly, as late as Dec. 11, when the last slide was used.

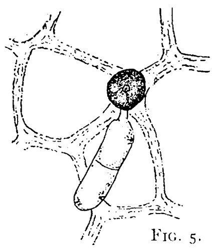

FIG. 5.

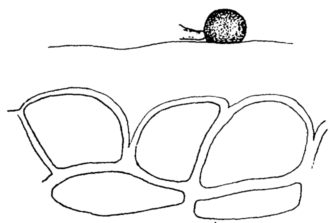

FIG. 7.

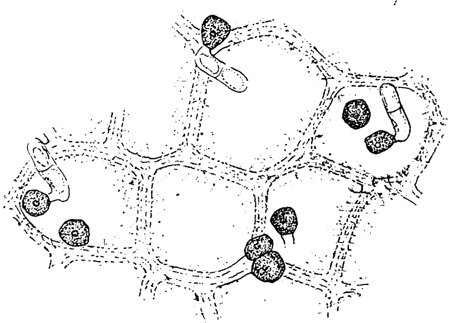

FIG. 6.

FIGS. 5, 6.—Natural appressoria formed on the surface of apples.

FIG. 7.—Section showing relation of adhesion organ to cuticle of apple.

During the hot summer weather the bitter-rot spores germinate immediately, and in 12–24 hours the appressoria are formed. Under natural conditions the germ tube is extremely short, since it immediately proceeds to the formation of an adhesion-disc. From this firmly adherent and more resistant organ the infecting hypha dissolves its way into the fruit. In badly infected trees the appressoria can often be found in great numbers adhering to the surface of the

apples. Such naturally formed appressoria are shown in *figs. 5, 6*, while in *fig. 7* a single adhesion organ is shown in section.

CONCLUSIONS.

The spore-like organs formed by the germ tubes of the anthracnoses are adhesion organs, by means of which the fungus is attached to the surface of its host during the early stages of infection. They are not suited for dissemination and therefore are not to be regarded as spores. The adhesion discs are formed as a result of stimuli from mechanical contact acting on the germ tubes. When growing in nutrient media the germ tubes lose their power of reacting to contact stimuli by the formation of appressoria. Under natural conditions the appressoria are formed as soon as the germ tube emerges from the spore.

NOTE.—In the same year ATKINSON describes these bodies for a species of Colletotrichum (*C. Gossypii*)[15] and suggests that their production in unfavorable conditions seems to favor the notion that they are resting bodies.

[15] ATKINSON, G. F., Anthracnoses of cotton. Journ. Mycol. **6**:173–8. *pls. 2.* 1891.

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